

# Influence of *in vitro* irrigation protocols on root canal treatment for bond strength to enamel and coronary dentin

Vanessa Lessa Araújo<sup>1</sup>, Rafaella Fontes Bragança<sup>1</sup>, Mario Torigoe<sup>1</sup>, Sheyn Akari Yamakami<sup>1,2</sup>, Hiroe Ohyama<sup>2</sup>, Juliana Jendiroba Faraoni<sup>1</sup>, Regina Guenka Palma-Dibb<sup>1</sup>

<sup>1</sup>Department of Restorative Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil, <sup>2</sup>Department of Restorative Dentistry and Biomaterials Sciences, Harvard School of Dental Medicine, Harvard University, Boston, MA, United States

## Abstract

**Introduction:** The purpose of this study was to evaluate the effects of different concentrations of chitosan solution used as a final rinse after root canal treatment on the shear bond strength of composite resin to enamel and coronary dentin.

**Materials and Methods:** Sixty-four enamel/dentin fragments were taken from the cervical-third of bovine teeth. The specimens were randomly divided into four groups according to the irrigation protocol (0.2% chitosan, 0.5% chitosan, 1% chitosan, and 17% ethylenediaminetetraacetic acid [EDTA]-control) and in two subgroups according to the substrate (enamel or dentin). Each specimen was fixed in a metallic clamping device and composite resin (Single Bond/z350) was inserted in two increments and polymerized for 20s. The microshear bond strength was performed, and the adhesive interface was analyzed by three-dimensional laser confocal scanning microscopy. The data were analyzed using a two-way ANOVA and Tukey's test ( $\alpha = 5\%$ ).

**Results:** For the irrigation protocol, bond strength was significantly higher for all chitosan solutions compared to the 17% EDTA-control solution. All chitosan solutions, 0.2%, 0.5% and 1.0%, were statistically similar to each other ( $P > 0.05$ ). For the substrate, enamel showed greater bond strength and was significantly different from dentin ( $P < 0.05$ ). For interaction factor, 1% chitosan solution presented bond strength values statistically superior to the 17% EDTA control solution ( $P < 0.05$ ) in dentin. The adhesive fracture was the most prevalent for all groups.

**Conclusion:** The irrigation protocol on the root canal treatment does not affect the enamel bond strength and 1% chitosan solution improved dentin bond strength. Bond strength was higher for enamel than for coronary dentin.

**Keywords:** Chitosan, composite resin, dentin, enamel, root number, shear strength

**Address for correspondence:** Dr. Regina Guenka Palma-Dibb, Department of Restorative Dentistry, FORP/USP, Av. do Café, s/nº, Monte Alegre - 14040-904, Ribeirão Preto, SP, Brazil.

E-mail: rgpalma@usp.br

**Submission:** 19-03-20 **Revision:** 16-05-20 **Acceptance:** 24-05-20 **Web Publication:** 08-05-21

## INTRODUCTION

The success of root canal treatment is directly associated with infection control<sup>[1]</sup> and final restoration sealing.<sup>[2]</sup>

Irrigating solutions are used not only as antimicrobial agents but also to lubricate dentin walls and to dissolve organic and inorganic components of the smear layer, favoring

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow\_reprints@wolterskluwer.com

**How to cite this article:** Araújo VL, Bragança RF, Torigoe M, Yamakami SA, Ohyama H, Faraoni JJ, *et al.* Influence of *in vitro* irrigation protocols on root canal treatment for bond strength to enamel and coronary dentin. Saudi Endod J 2021;11:181-7.

### Access this article online

Quick Response Code:



Website:

www.saudiendodj.com

DOI:

10.4103/sej.sej\_54\_20

the sealer infiltration in the dental structure.<sup>[3]</sup> Therefore, irrigating solutions play an important role in compensating for instrumentation shortcomings and complementing disinfection procedures.<sup>[4]</sup> Despite the beneficial action of the irrigants, these solutions can modify the dental surface structure, and consequently, have an adverse impact on the dentin properties.<sup>[5]</sup>

Sodium hypochlorite (NaOCl) solution is the most widely used root canal irrigant<sup>[1,6]</sup> due to its ability to eliminate the pathogenic microorganisms.<sup>[7]</sup> This antimicrobial activity can occur directly by a chemical effect and/or indirectly through facilitating mechanical debridement, dissolving tissue, and flushing off contaminated debris accumulated during canal preparation.<sup>[8,9]</sup> However, NaOCl is not able to dissolve inorganic dentin particles and prevent smear layer formation after canal instrumentation.<sup>[10]</sup> Therefore, chelating agents such as ethylenediaminetetraacetic acid (EDTA) are used as the final irrigant to demineralized dentin and to properly clean the root canal walls<sup>[11]</sup> complementing the action of NaOCl. EDTA improves the penetration of the filling material and increases contact with dentin walls, resulting in a better sealing.<sup>[12,13]</sup> However, EDTA has limited antiseptic capacity<sup>[3]</sup> and is neither biodegradable nor biocompatible.<sup>[14]</sup>

Chitosan solution was proposed as an alternative to EDTA in the root canal treatment<sup>[15,16]</sup> because of its favorable properties of biodegradability, biocompatibility, nontoxicity, and antimicrobial action.<sup>[17-20]</sup> It is a natural polymer obtained through the deacetylation of chitin found in arthropod shells<sup>[17]</sup> and possesses comparable chelating activity to EDTA.<sup>[15]</sup> Since chitosan is a linear biopolymer of glucosamine with a  $\beta$ -(1-4) linkage, it can improve collagen mechanical properties<sup>[18]</sup> through the carboxyl and hydroxyl groups present in its structure which confers surface hydrophilicity, polarity, and polyanionic nature, responsible for the sequestering sites configuration and favorable nucleation of calcium.<sup>[19]</sup> However, there are few studies that correlate chitosan influence on the adhesion of the composite resin to on the dentin surface.

The restoration process is crucial to re-establish teeth esthetics and functionality. It also prevents bacterial infiltration in the oral cavity, protecting remaining dental structure, and ensuring success of the technique.<sup>[2]</sup> Chemical substances used during biomechanical preparation of root canals also come into contact with the coronal surface.<sup>[21]</sup> As a result, dentin and enamel composition may be altered because of their interaction with restorative materials which can impair the final restoration sealing.<sup>[22]</sup> Therefore, the aim of this study was to evaluate the influence of different

concentrations of chitosan solution used as final rinse after the root canal treatment on the shear bond strength of composite resin to the coronary dentin/enamel.

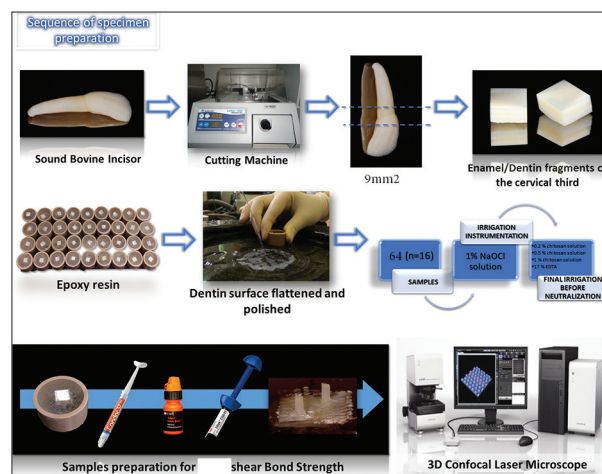
## MATERIALS AND METHODS

### Experimental design

This study followed a  $2 \times 4$  factorial design. The factors under investigation were the tooth substrate in two levels (enamel and dentin) and the final irrigation protocol in four levels (0.2% chitosan solution, 0.5% chitosan solution, 1% chitosan solution, and 17% EDTA control solution). The experimental units were composed of 64 enamel and dentin fragments obtained from bovine incisors ( $n = 64$ ). These 64 fragments were randomly assigned into four groups ( $n = 16$ ) and submitted to a microshear bond strength test (BS) at a 0.5 mm/s crosshead speed/GC 200 N applied at the resin-dentin/enamel interface. The response variables consisted of microshear bond strength evaluated in MPa and the failure pattern qualitatively analyzed by laser confocal microscopy.

### Specimen preparation

One hundred and twenty-eight [Figure 1] bovine incisors were freshly extracted and stored in deionized water at 4°C (pH = 7.0). They were then cleaned with a scaler and water/pumice slurry in dental prophylactic cups to remove all tissue remnants. After, the teeth were sectioned 2 mm below the cemento-enamel junction with a water-cooled diamond saw in a sectioning machine (Isomet 1000, Buehler, Lake Bluff, IL, USA). The crowns were sectioned longitudinally into buccal and palatal halves to obtain the enamel and dentin fragments to a size of 3 mm  $\times$  3 mm of the cervical third of the bovine incisors. Each specimen was embedded in



**Figure 1:** Sequence of specimen's preparation subjected to the evaluation of the influence of different concentrations of chitosan solution used as the final rinse after root canal treatment on the shear bond strength of the composite resin to coronary dentin/enamel

a transparent epoxy resin (Epoxicure; Bueher, São Paulo, SP, Brazil) with the enamel/dentin surface facing upward. After resin polymerization, their surfaces were flattened and polished with a #400-grit silicon carbide (SiC) paper (Hermes Abrasives Ltd., Virginia Beach, VA, EUA). To produce a standardized smear layer, the specimens were also polished with #600-grit SiC paper for 30 s in a polishing machine (Arotec, Cotia, SP, Brazil).

### Irrigation protocol

A #45 K-file (Kerr Corporation, Visé, Belgique) was used only on dentin surfaces in continuous and uniform back and forth movements with constant pressure to simulate root canal treatment, followed by irrigation with 1 mL of 1% NaOCl. Dentin specimens were randomly divided into four groups based on final solution used: 0.2% chitosan, 0.5% chitosan, 1% chitosan, and 17% EDTA (control). Chitosan solutions (pH 3.25) were obtained by diluting 0.2 g, 0.5 g, and 1.0 g of chitosan (low-molecular weight, 90% deacetylated, Sigma-Aldrich Brazil Ltda., São Paulo, Brazil) in 1 mL of 1% acetic acid solution. Irrigation protocol was performed with: (1) 1 mL of chitosan solution for 3 min, (2) followed by final root canal rinsing with 1 mL of 1% NaOCl to neutralize the active properties of chitosan, (3) and finally, the surface of the canal was dried with absorbent paper (Dentsply Maillefer, Petrópolis, Brazil). The same protocol was used for enamel specimens.

### Samples preparation for microshear bond strength

To perform the adhesive protocol, a bonding site was demarcated and a piece of Tygon “flexible silicone tube” with a 0.8 mm diameter central hole was attached to the surface of each specimen. The delimited delineated area of each specimen was etched with 35% phosphoric acid for 30 s (enamel) and 15 s (dentin), then washed with distilled water and dried with absorbent paper. Two coats of adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA) were applied and dried with a brief, mild air stream, according to the manufacturer’s instructions. Then, the adhesive agent was light cured for 20 s.

Specimens were individually fixed in a metallic clamping device (developed by Houston Biomaterials Research Center and manufactured at the Precision Workshop from University of São Paulo). Composite resin (Filtek Z250, 3M ESPE, St. Paul, MN, USA) was inserted into the polyethylene tubing (Tygon, Norton Performance Plastic Co, Cleveland, OH, USA) with a 2 mm internal diameter  $\times$  4 mm height, in two increments, and light cured for 20 s. The specimens were detached from the clamping device, and the tygon was removed with an n.15 scalpel blade (Med Blade) leaving a composite resin

cylinder (2 mm diameter  $\times$  4 mm high) adhered to the delimited dentin surface. The samples were stored in 100% relative humidity at 37°C for 24° h until the bond strength performance test.

The specimens were subjected to a microshear test in a universal testing machine (Instron Corporation, Canton-Massachusetts, USA). The test was performed at a crosshead speed of 0.5 mm/s with GC 200 N until failure fracture occurred. The failure load in N was divided by the bonded area in mm to obtain the shear bond strength in Megapascal (MPa). After, the failure pattern of the specimens were analyzed by three-dimensional (3D) Laser Confocal Microscopy (LEXT, 3D Measuring Laser Microscope OLS 4000, Olympus Corporation) and classified as: (i) adhesive failure (dentin surface covered by thin layer of adhesive material), (ii) cohesive material failure (surface covered by composite resin), (iii) cohesive substrate failure (when failure occurs in the enamel/dentin), and (iv) mixed failure (combination of adhesive and cohesive types).

### Statistical analysis

After evaluating the assumptions of normality and homoscedasticity, collected data were subjected to a two-way ANOVA considering tooth substrate and irrigation solution as the independent variables. Multiple comparisons were performed with a *post hoc* Tukey’s test ( $\alpha = 5\%$ ). Statistical calculations were performed using the SPSS software version 19 (SSPSS Inc., Chicago, IL, USA).

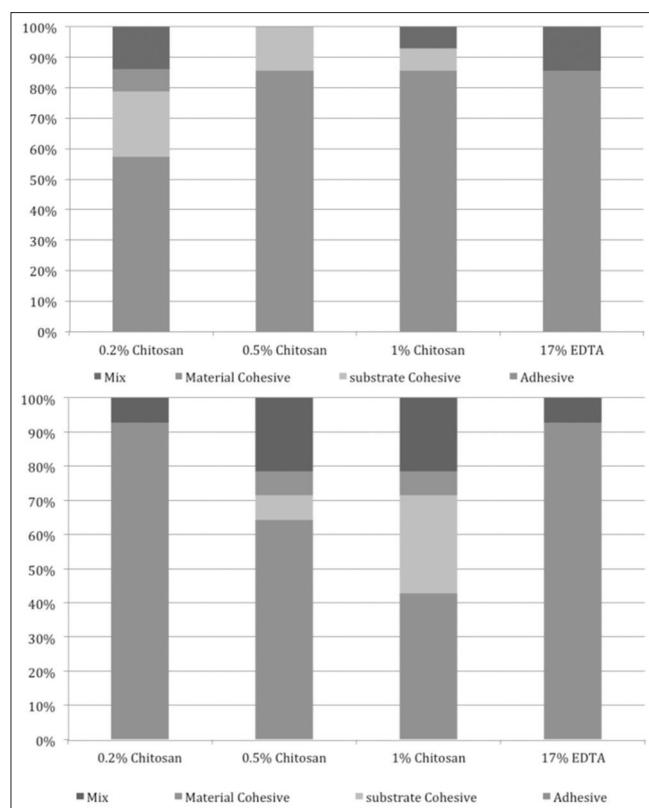
## RESULTS

For the microshear test, data analysis showed that the irrigation protocol factor presented a significant difference among the groups for substrates, enamel, and dentin ( $P < 0.05$ ). Bond strength was significantly higher for all chitosan solutions compared to the 17% EDTA control solution. All chitosan solutions, 0.2%, 0.5%, and 1.0% were statistically similar to each other ( $P > 0.05$ ). For the substrate factor, enamel showed higher bond strength and was significantly different from dentin ( $P < 0.05$ ) [Table 1].

Interaction between the factors irrigation protocol and substrate, the four solutions showed a similar effect and did not affected the bond strength to enamel ( $P > 0.05$ ). The bond strength to dentin was statistically superior for the 1% chitosan solution when compared to the 17% EDTA control solution.

According to the failure pattern analysis by 3D laser confocal microscopy [Figure 2], it was observed that adhesive fracture was the most prevalent in all groups for





**Figure 2:** Percentage of occurrence of the failure pattern according to the groups for both substrates. Upper means of failure patterns in enamel. Lower means of failure patterns in dentin

**Table 1: Mean and standard deviation of the microshear bond strength (MPa) according to substrate and irrigation solution**

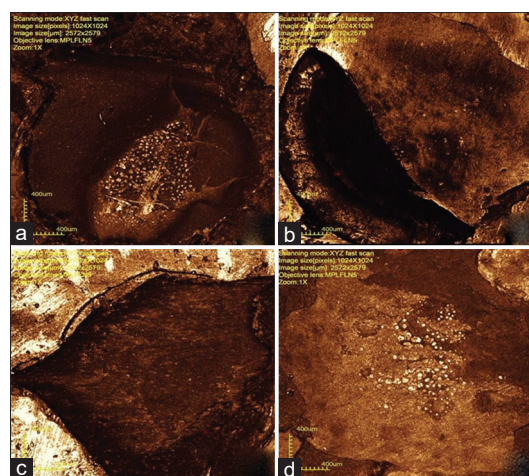
Irrigation solution	Mean±SD (MPa)	
	Enamel	Dentin
0.2% chitosan solution	20.44±7.04 <sup>a</sup>	16.59±5.48 <sup>ab</sup>
0.5% chitosan solution	21.01±6.45 <sup>a</sup>	16.39±5.74 <sup>ab</sup>
1.0% chitosan solution	18.95±3.91 <sup>a</sup>	19.27±4.59 <sup>a</sup>
17% EDTA solution (control)	16.41±6.33 <sup>a</sup>	11.12±4.57 <sup>b</sup>

Matching lowercase letters indicate statistical similarity ( $P < 0.05$ ) in the same column. EDTA: Ethylenediaminetetraacetic acid, SD: Standard deviation

both substrates varying between 40% and 90% for the different. Figure 3 presents the representative images for each failure pattern observed by laser confocal microscopy.

## DISCUSSION

Endodontic treatment triggers an imbalance between inorganic and organic compounds responsible for altering the ultrastructural and mechanical characteristics of the dental structure.<sup>[22,23]</sup> However, the impact of final irrigation protocol on the root canal treatment in the adhesion of restorative materials to the coronary structure is still unknown. Recent substances have been proposed to replace final irrigation, such as chitosan solution, which due to covalent immobilization to collagen can induce



**Figure 3:** Representation of failure type observed by laser confocal microscopy. (a) Cohesive material failure; (b) Mixed failure (combination of adhesive/cohesive material failure); (c) Cohesive substrate failure; (d) Mixed failure (combination of adhesive/cohesive substrate failure)

the remineralization of exposed tissue.<sup>[24]</sup> Therefore, the methodology employed in this study investigated the effects of different concentrations of chitosan solution used as a final rinse after the root canal treatment to improve the shear bond strength of composite restorations to the dental structure. For this, a filing simulation was performed on bovine enamel and dentin specimens, with the same movements performed in endodontic treatment and the irrigation protocols with the different solutions were performed. To evaluate the bond strength, the microshear test was employed to evaluate the interaction of the composite resin to the tooth structure and 3D laser confocal microscopy was used to analyze the failure pattern.

Of the various beneficial characteristics of chitosan, its exceptional efficacy as a root canal bactericidal agent has already been demonstrated.<sup>[25,26]</sup> This effect occurs due to its positive charge, which makes it able to adsorb the bacterial cell wall.<sup>[27]</sup> In addition, its low-molecular weight and high deacetylation degree promote bactericidal activity.<sup>[28,29]</sup> For this reason, in this study, we opted for chitosan with a low-molecular weight and 90% deacetylation to maximize the proven bactericidal property for the irrigation of contaminated root canals.<sup>[30]</sup>

As regard to chelating ability, chitosan can be characterized by  $\beta$ -(1-4)-linked 2-acetamido-2-deoxy- $\beta$ -D-glucopyranose and 2-amino-2-deoxy- $\beta$ -D-glycopyranose<sup>[29]</sup> in which the nitrogen atoms located in the structural chain are equipped with free-electron pairs involved in the ion exchange between metal ions and chelating agents. The chelation action, ion exchange, and adsorption properties are considered the mechanisms responsible for the complex arrangements between chitosan polymer and dentin.<sup>[31]</sup>

Although there are several studies that credit acetic acid solvent for chitosan's efficacy in dissolving calcium ion, recent evidence has shown that demineralization of extrafibrillary dentin is due to the chelating properties of chitosan rather than the action of the acetic acid.<sup>[29]</sup> For this reason, studies showed that root canal irrigation with chitosan solution had statistically significantly higher bond strength values than those irrigated with EDTA ( $P < 0.05$ ) and could, therefore, be responsible for the better sealing of root canals and long-term duration of endodontic treatments.<sup>[26]</sup>

Along with EDTA – the industry standard NaOCl is routinely employed as an irrigating solution to complement root canal wall cleansing (NaOCl-EDTA).<sup>[12,13]</sup> Moreover, NaOCl is used before final irrigation which can decrease mechanical resistance<sup>[32,33]</sup> and denaturation resulting in the dissolution of dentin collagen. It could also be responsible for causing ultrastructural damage to dentin substrate,<sup>[34]</sup> influencing hardness values,<sup>[35,36]</sup> flexural strength, and modulus of dentin elasticity<sup>[37,38]</sup> which were significantly reduced without NaOCl use. In fact, NaOCl can increase dentin surface roughness and create a space at the interface due to collagen depletion,<sup>[39]</sup> which may present a high risk for the occurrence of vertical root fractures in endodontically treated teeth.<sup>[18]</sup> As expected, in this study, the protocol (NaOCl-EDTA) presented the worst performance in relation to the bond strength, probably because the association of NaOCl combined with a chelating agent resulting in dentin demineralization, dentinal tubules enlargement, and softening and denaturation of dentin collagen fibers.<sup>[40-42]</sup> The NaOCl-EDTA-NaOCl irrigation sequence may destroy the protective hydroxyapatite capsule of collagen fibers. For this reason, this sequence shows a potential harmful influence on the adhesion of some resin sealers to the dentin structure,<sup>[43]</sup> besides reducing the bonding strength of some materials to dental crown.

In order to increase the durability of adhesive restorations in endodontically treated teeth, chitosan was employed at different concentrations (0.2%, 0.5%, and 1%) due to its ability to strengthen and stabilize the collagen structure by forming microfibrillary arrays with superior mechanical properties.<sup>[44]</sup> The different concentrations of chitosan were selected to test the most effective percentage capable of interacting with the dental substrate and forming a surface more resistant to degradation. This study showed that the irrigation protocol with chitosan solution was able to provide greater microshear bond strength for enamel/dentin groups compared to the EDTA control solution. Particularly, the 1% chitosan solution showed improved bond strength for the dentin substrate compared to the

EDTA control solution. This observation may be explained because the incorporation of chitosan into the dentin structure improves the biological and mechanical properties of collagen fibrils by forming a protective coating.<sup>[44,45]</sup> In this process, functional phosphate groups can bind to calcium ions forming a rich calcium phosphate layer with a surface conformation favorable to crystal nucleation. This layer may protect the overexposed collagen fibrils, and as a result, increase the dentin bond strength, especially if the effects of a long-term adhesive restoration are considered.<sup>[24]</sup>

To continue, chitosan has been used to stabilize the bonding resin-dentin interface during extrafibrillary dentin demineralization.<sup>[30]</sup> This strategy was developed to prevent the endogenous degradation of collagen that is initiated by the metalloproteinases (MMPs) present in saliva/dentin as well as to avoid water permeation in the dentin hybrid layer.<sup>[30,46]</sup> These characteristics may lead to collagen fibrils stabilization through an electrostatic mechanism that provides higher resistance to degradation and improved mechanical properties, forming a more resilient demineralized collagen substrate with a longer durability of the resin-dentin interface.<sup>[47,48]</sup> Neelakantan *et al.*<sup>[48]</sup> also found similar results, showing some level of affinity between chitosan and collagenase enzymes in which chitosan not only protected collagen fibrils and blocked access to MMPs but also impaired the MMPs activity. This could justify the better performance of chitosan in dentin than in enamel when considering the type of substrate.

In the present study, the enamel bond strength was greater as compared to the dentin substrate. This result can be clarified by analyzing each irrigation group, in which the bond strength to enamel was greater except for the 1% chitosan group which presented similar microshear bond strength values for enamel/dentin substrate, demonstrating that chitosan performance may be dependent on its concentration.<sup>[47]</sup> This observation becomes important in restorative dentistry. Although chitosan solution did not affect the enamel adhesion values, it allowed a better performance in dentin, especially with chitosan with a 1% concentration. While this may be true, the fracture mode analysis revealed that adhesive failure was the most prevalent fracture cause found in this study, and thus, chitosan did not affect the failure pattern in the specimens the exception for chitosan 1% in dentin that occurred increase of failure of substrate, probably due more interaction of chitosan with dentin. In fact, although chitosan groups presented better results than EDTA regarding the resin-dentin/enamel bond strength, the failure pattern did not change among the different treatments.

In conclusion, the ability of chitosan to bind to collagen fibers by electrostatic attraction may increase the resistance of exposed collagen and protect it against the actions of collagenase enzymes.<sup>[16,25]</sup> These factors are important when considering the durability of the restoration as a result of the preservation of collagen fibers in the mechanical imbrication of the bond system to the coronary dentin.<sup>[43-50]</sup> In fact, the use of chitosan as final irrigating solution was able to minimize the negative action of NaOCl and prevent damage to dentin by EDTA, resulting in the most optimal bioadhesion performance. However, it is important to emphasize that, as in all *in vitro* studies, they are performed in a controlled environment, but have limitations. In this case, although the entire methodology was designed as close as possible to the clinical situation, the teeth were not treated and exposed to situations corresponding to the challenges of the oral cavity. Therefore, within the limitations of an *in vitro* study, 1% chitosan proves to be an interesting alternative in the final rinse of the root canal treatment to improve the adhesion of dentin coronary, which is considered a critical factor for the success of the restorative technique. However, this study was tested only the immediate bond strength, suggesting that further research be performed on the longitudinal tests to establish the effectiveness of chitosan as a compound for irrigating solution in the endodontic treatment.

## CONCLUSION

The irrigation protocol of root canal treatment did not affect enamel bond strength, but in relation to dentin, 1% chitosan solution was able to improve the bond strength of the dentin structure.

## Acknowledgments

The authors have no conflicts of interest. This study was support by CNPq (311762/2013-1).

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Zeng C, Meghil MM, Miller M, Gou Y, Cutler CW, Bergeron BE, et al. Antimicrobial efficacy of an apical negative pressure root canal irrigation system against intracanal microorganisms. *J Dent* 2018;72:71-5.
- Farina AP, Cecchin D, Barbizam JV, Carlini-Júnior B. Influence of endodontic irrigants on bond strength of a self-etching adhesive. *Aust Endod J* 2011;37:26-30.
- Stelzer R, Schaller HG, Gernhardt CR. Push-out bond strength of RealSeal SE and AH Plus after using different irrigation solutions. *J Endod* 2014;40:1654-7.
- Almeida G, Marques E, De Martin AS, da Silveira Bueno CE, Nowakowski A, Cunha RS. Influence of irrigating solution on postoperative pain following single-visit endodontic treatment: Randomized clinical trial. *J Can Dent Assoc* 2012;78:c84.
- Gu LS, Huang XQ, Griffin B, Bergeron BR, Pashley DH, Niu LN, et al. Primum non nocere - The effects of sodium hypochlorite on dentin as used in endodontics. *Acta Biomater* 2017;61:144-56.
- Basudan SO. Sodium hypochlorite use, storage, and delivery methods: A survey. *Saudi Endod J* 2019;9:27-33.
- Divia AR, Nair MG, Varughese JM, Kurien S. A comparative evaluation of *Morinda citrifolia*, green tea polyphenols, and Triphala with 5% sodium hypochlorite as an endodontic irrigant against *Enterococcus faecalis* An *in vitro* study. *Dent Res J (Isfahan)* 2018;15:117-22.
- Kokkas AB, Boutsoukis ACh, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: An *in vitro* study. *J Endod* 2004;30:100-2.
- Vilanova WV, Carvalho-Junior JR, Alfredo E, Sousa-Neto MD, Silva-Sousa YT. Effect of intracanal irrigants on the bond strength of epoxy resin-based and methacrylate resin-based sealers to root canal walls. *Int Endod J* 2012;45:42-8.
- Paqué F, Rechenberg DK, Zehnder M. Reduction of hard-tissue debris accumulation during rotary root canal instrumentation by etidronic acid in a sodium hypochlorite irrigant. *J Endod* 2012;38:692-5.
- Butala R, Kabbinala P, Ballal V. Comparative evaluation of ethylenediaminetetraacetic acid, maleic acid, and peracetic acid in smear layer removal from instrumented root canal system: A scanning electron microscopic analysis study. *Saudi Endod J* 2017;7:170-5.
- Teixeira CS, Felipe MC, Felipe WT. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: An SEM analysis. *Int Endod J* 2005;38:285-90.
- Kishen A, Shrestha A, Del Carpio-Perochena A. Validation of Biofilm Assays to Assess Antibiofilm Efficacy in Instrumented Root Canals after Syringe Irrigation and Sonic Agitation. *J Endod* 2018;44:292-8.
- Spanó JC, Silva RG, Guedes DF, Sousa-Neto MD, Estrela C, Pécora JD. Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. *J Endod* 2009;35:727-30.
- Silva PV, Guedes DF, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: A new solution for removal of smear layer after root canal instrumentation. *Int Endod J* 2013;46:332-8.
- Hashmi A, Zhang X, Kishen A. Impact of dentin substrate modification with chitosan-hydroxyapatite precursor nanocomplexes on sealer penetration and tensile strength. *J Endod* 2019;45:935-42.
- Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules* 2003;4:1457-65.
- Shrestha A, Friedman S, Kishen A. Photodynamically crosslinked and chitosan-incorporated dentin collagen. *J Dent Res* 2011;90:1346-51.
- Chen Z, Cao S, Wang H, Li Y, Kishen A, Deng X, et al. Biomimetic remineralization of demineralized dentine using scaffold of CMC/ACP nanocomplexes in an *in vitro* tooth model of deep caries. *PLoS One* 2015;10:e0116553.
- Babu B, Nair RS, Angelo JM, Mathai V, Vineet RV. Evaluation of efficacy of chitosan-silver nanocomposite on *Candida albicans* when compared to three different antifungal agents in combination with standard irrigation protocol: An *ex vivo* study. *Saudi Endod J* 2017;87-91.
- Santos JN, Carrilho MR, De Goes MF, Zaia AA, Gomes BP, Souza-Filho FJ, et al. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. *J Endod* 2006;32:1088-90.
- Arslan S, Balkaya H, Çakir NN. Efficacy of different endodontic irrigation protocols on shear bond strength to coronal dentin. *J Conserv Dent* 2019;22:223-7.



23. Soares CJ, Rodrigues MP, Faria-E-Silva AL, Santos-Filho PC, Veríssimo C, Kim HC, et al. How biomechanics can affect the endodontic treated teeth and their restorative procedures? *Braz Oral Res* 2018;32:169-83.
24. Xu Z, Neoh KG, Lin CC, Kishen A. Biomimetic deposition of calcium phosphate minerals on the surface of partially demineralized dentine modified with phosphorylated chitosan. *J Biomed Mater Res B Appl Biomater* 2011;98:150-9.
25. Madhavan K, Belchenko D, Motta A, Tan W. Evaluation of composition and crosslinking effects on collagen-based composite constructs. *Acta Biomater* 2010;6:1413-22.
26. Daood U, Igbal K, Nitisusanta LI, Fawzy AS. Effect of chitosan/riboflavina modification on resin/dentin interface: Spectroscopic and microscopic investigations. *J Biomed Mater Res A* 2013;101:1846-56.
27. Shrestha A, Hamblin MR, Kishen A. Photoactivated rose bengal functionalized chitosan nanoparticles produce antibacterial/biofilm activity and stabilize dentin-collagen. *Nanomedicine* 2014;10:491-501.
28. Fujiwara M, Hayashi Y, Ohara N. Inhibitory effect of watersoluble chitosan on growth of *Streptococcus mutans*. *New Microbiol* 2004;27:83-6.
29. Tachaboonyakiat W. Antimicrobial applications of chitosan. *Chitosan based. Biomaterials* 2017;2:245-74.
30. Ozlek E, Rath PP, Kishen A, Neelakantan P. A chitosan-based irrigant improves the dislocation resistance of a mineral trioxide aggregate-resin hybrid root canal sealer. *Clin Oral Investig* 2020;24:151-6.
31. No HK, Park NY, Lee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int J Food Microbiol* 2002;74:65-72.
32. Madhusudhana K, Satyavathi E, Lavanya A, Suneelkumar C, Deepthi M. Research article comparison of the effect of chitosan and morindacitrifolia on smear layer removal: An *in vitro* study. *Sch J Dent Sci* 2015;2:132-6.
33. Gu LS, Cai X, Guo JM, Pashley DH, Breschi L, Xu HH, et al. Chitosan-based extrafibrillar demineralization for dentin bonding. *J Dent Res* 2019;98:186-93.
34. Dasha MF, Chiellini RM, Ottenbriteb E. Chitosan-A versatile semi-synthetic polymer in biomedical applications. *Prog Polym Sci* 2011;36:981-1014.
35. Gusiyska A, Dyulgerova E, Vassileva R, Gyulbenkiyan E. The effectiveness of chitosan-citrate solution to remove the smear layer in root canal treatment – An *in vitro* study. *Int J Sci Res* 2016;5:1169-74.
36. Gu L, Mazzoni A, Gou Y, Pucci C, Breschi L, Pashley DH, et al. Zymography of hybrid layers created using extrafibrillar demineralization. *J Dent Res* 2018;97:409-15.
37. Fawzy AS, Nitisusanta LI, Igbal K, Daood U, Beng LT, Neo J. Chitosan/Riboflavin-modified demineralized dentin as a potential substrate for bonding. *J Mech Behav Biomed Mater* 2013;17:278-89.
38. Persadmehr A, Torneck CD, Cvitkovitch DG, Pinto V, Talior I, Kazembe M, et al. Bioactive chitosan nanoparticles and photodynamic therapy inhibit collagen degradation *in vitro*. *J Endod* 2014;40:703-9.
39. Niu W, Yoshioka T, Kobayashi C, Suda H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. *Int Endod J* 2002;35:934-9.
40. Gringoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentin to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int Endod J* 2001;34:113-9.
41. Slutzky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. *J Endod* 2004;30:880-2.
42. Oliveira LD, Carvalho CA, Nunes W, Valera MC, Camargo CH, Jorge AO. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104:e125-8.
43. Marending M, Paqué F, Fischer J, Zehnder M. Impact of irrigant sequence on mechanical properties of human root dentin. *J Endod* 2007;33:1325-8.
44. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. *J Endod* 2002;28:17-9.
45. Kishen A. Mechanisms and risk factors for fracture predilection in endodontically treated teeth. *Endod Topics* 2006;13:57-83.
46. Tay FR, Pashley DH, Loushine RJ, Weller RN, Monticelli F, Osorio R. Self-etching adhesives increase collagenolytic activity in radicular dentin. *J Endod* 2006;32:862-8.
47. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjäderhane L, Mazzoni A, et al. Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. *J Biomed Mater Res B Appl Biomater* 2009;90:373-80.
48. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentin conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. *Int Endod J* 2011;44:491-8.
49. Pascon FM, Kantovitz KR, Sacramento PA, Nobre-dos-Santos M, Puppini-Rontani RM. Effect of sodium hypochlorite on dentin mechanical properties. A review. *J Dent* 2009;37:903-8.
50. Neelakantan P, Sharma S, Shemesh H, Wesselink PR. Influence of irrigation sequence on the adhesion of root canal sealers to dentin: A fourier transform infrared spectroscopy and push-out bond strength analysis. *J Endod* 2015;41:1108-11.